FINAL REPORT

GRANT #: N00014-02-1-0957

PRINCIPAL INVESTIGATOR: Dr. Lan Bo Chen

INSTITUTION: Shionogi BioResearch Corp./Synta Pharmaceuticals Corp.

GRNAT TITLE: Standoff Biological Threat Agent Monitoring and Neutralization

AWARD PERIOD: 1 June 2002 – 30 June 2003

OBJECTIVES: The long range goal is to detect air-born anthrax spores by standoff acoustic means. The short-term objective is to identify fluorescent dyes that can bind to or react quickly with bacterial spores similar to anthrax spores.

APPROACH: B. subtilis spores (which have surface properties similar to that of anthrax spores) were used in high through-put screenings against a large collection of fluorescent dyes for fast binders with high affinity or covalent-bonders. It is hoped that the attachment of organic molecules to spores will allow rapid standoff detection by acoustic method developed at Los Alamos by Dr. Dipen Sinha..

ACCOMPLISHMENTS: B. subtilis was grown in medium optimal for exponential growth. Once reach stationary phase, it was harvested and placed in nutrients-deprived medium optimal for spore formation. Spores were recovered and plated at 10,000 spores per well in 96-well plates or 384-well plates with V-shape bottom appropriate for centrifugation. Fluorescent dyes were dissolved in DMSO at 1 mg/ml as stock solution. Each dye was added in duplicate wells at 10 ug/ml in spore promoting medium and incubated for 30 min. Plates were centrifuged for 5 min at 1,000xG and spores resuspended in spore medium, followed by recentrifugation. This sequence was repeated twice to remove unbound fluorescent dyes. Fluorescence associated with spores was measured by plate reader at four different filter sets (normally used for rhodamine dye, fluorescein dye, Texas Red dye or DAPI dye) to cover essentially all excitation and emission wavelengths of the dyes in our chemical library.

Dyes able to associate with B. subtilis spores in the above screens were selected and subjected to the following test. About 100,000 spores in spore medium were placed in culture conical centrifuge tube. Fluorescent dye was added at 10 ug/ml and incubated at room temperature for 30 min. Spores were recovered by centrifugation at 2,000 x G and resuspended in dye-free spore medium. This process was repeated twice to remove excess dyes. Spores were then resuspended in 10 ul aliquot and placed under microscope slides for examination by fluorescent microscope. Whether fluorescent dyes are unambiguously associated with spores was examined and confirmed by laser scanning confocal fluorescent microscope.

CONCLUSIONS: About 25,000 fluorescent dyes were screened. Thirty two dyes were positive in the initial screenings and 14 dyes were confirmed by laser confocal microscope. Five dyes were chosen for further examination to be carried out in the next phase of work. We plan to measure binding kinetics and affinity constant, to determine the nature of binding, and, to examine shift in fluorescent spectra upon binding.

SIGNIFICANCE: The fluorescent dyes that bind B. subtilis spores identified here may not only be potentially useful for detection of anthrax spores but also serve as leads for new class of antibiotics that can prevent spore from germination. Spore-neutralizing pharmaceuticals will be welcomed in both civilian and military applications.

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14. ABSTRACT

Our objective is to identify fluorescent dyes that can bind to or react quickly with bacterial spores including anthrax spores. B. subtilis spores (which have surface properties similar to that of anthrax spores) were used in screenings against a collection of fluorescent dyes for fast binders with high affinity or for covalent-bonders. It is hoped that the attachment of organic molecules to spores will allow rapid standoff detection by acoustic method developed at Los Alamos by Dr. Dipen Sinha. Thirty two dyes were positive in the initial screenings and 14 dyes were confirmed by laser confocal microscopy. Five dyes have been chosen for the next phase of work including the measurement of binding kinetics and affinity constant, the determination of the nature of binding, and, the measurement of shift in fluorescent spectra upon binding.

15. SUBJECT TERMS

Anthrax spores, standoff detection, fluorescent dyes, acoustics, microscopy.

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